FORM-PTO-1390 (Rev. 12-29-99)

U S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 033312-001

U S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/830432 Unassigned

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/00990	28 August 2000	26 August 1999

TITLE OF INVENTION: COMPOSITION COMPRISING MICRONUTRIENTS IN COMBINATION WITH PREBIOTICS, PROBIOTICS, AND/OR SYNBIOTICS

APPLICANT(S) FOR DO/EO/US: Stanley H. ZLOTKIN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

531 Rec'd PCT 26 APR 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5(b))	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Unassigned 09/830432	PCT/CA00/00990	033312-001

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	25 -20 =	5	X$18.00 (966)	$ 90.00	
Independent Claims	5 -3 =	2	X$80.00 (964)	$ 160.00	
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1,110.00	
Reduction for 1/2 for filing by small entity, if applicable (see below).				$ 0.00	-
SUBTOTAL =				$ 1,110.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0.00	
TOTAL NATIONAL FEE =				$ 1,110.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$ 0.00	
TOTAL FEES ENCLOSED =				$ 1,110.00	
				Amount to be: refunded	$
				charged	$

a. ☐ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 1,110.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Date: April 26, 2001

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26,999
REGISTRATION NUMBER

Patent
Attorney's Docket No. 033312-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Stanley H. ZLOTKIN	)	Group Art Unit: Unassigned
	)	
Application No.: Corresponds to International Application No. PCT/CA00/00990	)	Examiner: Unassigned
	)	
International Application Filed: August 28, 2000	)	
	)	
For: COMPOSITION COMPRISING MICRONUTRIENTS IN COMBINATION WITH PREBIOTICS, PROBIOTICS, AND/OR SYNBIOTICS	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Preliminary to examination of the above-captioned patent application, kindly amend the application in the following manner.

IN THE CLAIMS:

Kindly replace Claims 5, 8, 10, 14, 15 and 20 as follows.

5. (Amended) The composition as in claim 3 wherein the composition additionally comprises FeNaEDTA.

8. (Amended) The composition as in claim 1, wherein the prebiotic is selected from at least one member of the group consisting of FOS, inulin, GOS, lactulose, and lactitol.

10. (Amended) The composition as in claim 1, wherein the probiotic is selected from at least one member of the group consisting of Lactobacilli, Gram-positive cocci, and Bifidobacteria.

14. (Amended) the composition as in claim 1, wherein the synbiotic is selected from at least one member of the group consisting of Bifidobacteria + FOS, Lactobacilli + lactitol, and Bifidobacteria + GOS.

15. (Amended) The composition as in claim 1, wherein at least one micronutrient is microencapsulated with a compound selected from the group consisting of monoglycerides, diglycerides, ethyl cellulose, hydrogenated soybean oil and mixtures thereof.

20. (Amended) Use of the composition of claim 1 for enhancing the general immunity of a mammal.

REMARKS

The wording in Claims 5, 8, 10, 14, 15 and 20 has been amended to delete the multiple dependency. No new matter has been introduced.

These changes have been made in accordance with 37 C.F.R. § 1.121 as amended on November 7, 2000. Marked-up versions of Claims 5, 8, 10, 14, 15 and 20 indicating the changes accompany this Preliminary Amendment.

Early and favorable consideration with respect to this application is respectfully requested.

Should any questions arise in connection with this application, the undersigned respectfully requests that he be contacted at the number indicated below.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

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Date: April 26, 2001

Attachment to Preliminary Amendment dated April 26, 2001

Marked-up Claims 5, 8, 10, 14, 15 and 20

5. (Amended) The composition as in [any of claims 3 and 4] claim 3 wherein the composition additionally comprises FeNaEDTA.

8. (Amended) The composition as in [any of claims 1-7,] claim 1, wherein the prebiotic is selected from at least one member of the group consisting of FOS, inulin, GOS, lactulose, and lactitol.

10. (Amended) The composition as in [any of claims 1-7,] claim 1, wherein the probiotic is selected from at least one member of the group consisting of Lactobacilli, Gram-positive cocci, and Bifidobacteria.

14. (Amended) the composition as in [any of claims 1-13,] claim 1, wherein the synbiotic is selected from at least one member of the group consisting of Bifidobacteria + FOS, Lactobacilli + lactitol, and Bifidobacteria + GOS.

15. (Amended) The composition as in [any of claims 1-14,] claim 1, wherein at least one micronutrient is microencapsulated with a compound selected from the group consisting of monoglycerides, diglycerides, ethyl cellulose, hydrogenated soybean oil and mixtures thereof.

Attachment to Preliminary Amendment dated April 26, 2001

Marked-up Claims 5, 8, 10, 14, 15 and 20

20. (Amended) Use of the composition of [any of claims 1-17] claim 1 for enhancing the general immunity of a mammal.

COMPOSITION COMPRISING MICRONUTRIENTS IN COMBINATION WITH PREBIOTICS, PROBIOTICS, AND/OR SYNBIOTICS

Field of the Invention

The present invention relates to supplements for enhancement of the immune system. More particularly, the present invention relates to compositions combining micronutrients, probiotics, prebiotics, and synbiotics which are especially useful for enhancement of the immune system.

Background of the Invention

Proper nutrition is critical to the development of an effective immune system and enhancement of the natural immunosurveillance immune effector mechanism. This enhancement could be mediated either by increasing the frequency and absolute numbers of effector cells that carry out such function or by enhancement of the cellular mechanisms by which such effector cells mediate their function.

The clinical association of particular importance is between malnutrition and an individual's ability to respond to infectious micro-organisms or their antigenic constituents. Mechanisms by which nutrition affects immunity include reduced phagocytic activity and decreased leukocyte proliferation which, respectively, result in less vigorous microbial elimination and poor clonal expansion of microbe-specific lymphocytes. In addition, cell cycle, transcription regulation, antibody production, cytokine secretion and anti-oxidant protection may also be altered. Thus, the immune problems related to nutritional deficiencies vary from increased opportunistic infections to suboptimal responses following vaccination. In such cases dietary supplementation of micronutrients is likely to enhance immune function.

One type of malnutrition is micronutrient malnutrition, which may be defined as the insufficient dietary consumption of nutrients such as vitamin A, zinc, iron and iodine. It is a significant problem affecting more than 2 billion people worldwide, particularly women and children living in poverty. Iron deficiency is the most common nutritional problem in the world, affecting two thirds of children in most developing nations. The consequences of iron deficiency anemia are very serious. Anemia resulting from iron deficiency in young children has become very common since the level of bioavailable iron in a typical infant's diet is low while their rapid growth requires a much higher level of iron. The consequences of iron deficiency anemia (IDA) are very serious as it is associated with impaired cognitive and psychomotor development, reduced growth and decreased resistance to infection. Recent demonstration that these negative effects seem not to be reversible, at least until school entry, has significant public health implications. In January 1995, the WHO/UNICEF Joint Committee on Health Policy decided that iron supplement programs for the prevention of iron deficiency anemia should include infants and children from six months to five years of age and all low birth-weight infants from three to 12 months.

Vitamin A and its derivatives are important, not only for normal functioning of the eye, but also for normal differentiation of several tissues. Vitamin A is also an essential micronutrient needed in small amounts for normal functioning of the visual system, growth and development, maintenance of epithelial cell integrity, immune function, and reproduction. In the vitamin A deficient state, the human is unable to raise an adequate antibody response to bacteria and to maintain the activity and number of killer cells. There is documentation, for example, that mucosal immune response to cholera toxin is impaired. Vitamin A also plays a role in the production of cell glycoprotein and in the regulation of cell division in the intestine which has a bearing on intestinal epithelial renewal during and after acute enteric infections. An association between vitamin A deficiency and increased diarrhea morbidity has been reported. Vitamin A supplementation has been shown to decrease the mortality from

diarrhea and measles. Since the 'defining' studies of Findlay and Mackenzie in the early 1920's, several reports have suggested an interaction between vitamin A and iron metabolism. These early studies demonstrated a reduction in hematopoietic cells in bone marrow and hemosiderosis in the liver and spleen in vitamin A deficient subjects. These and later studies also suggested that the lack of vitamin A may lead to a mild anemia characterized by low serum Fe and elevated level of Fe in storage depots, especially in the liver. An array of epidemiological studies indicated that vitamin A deficiency and anemia often coexist.

Zinc is another nutritionally essential micronutrient for humans. The zinc atom has a unique combination of properties that renders it useful in biologic systems. Zinc is an essential component of more than 200 enzymes pervading all metabolic pathways. The role of zinc in such enzymes can be structural and catalytic. Zinc is essential for cell growth and has a fundamental role in gene replication, activation, repression, transcription and translation.

The biologic actions of zinc have an important bearing on various components of the immune system. Zinc deficiency, both acquired and inherited, is associated with lymphoid atrophy, decreased cutaneous delayed hypersensitivity responses, lower thymic hormone activity, a decreased number of antibody-forming cells and impaired T-killer-cell activity. Reduced activity of thymic hormone which is involved in the differentiation of T cells has also been described in zinc deficiency.

Bhan et al.[1] describe the role of Zinc and Vitamin A supplementation for the prevention of diarrhea caused by malnutrition. Sazawal et al.[2] evaluated the effect of daily supplementation with 10 mg of elemental zinc on the incidence and prevalence of acute lower respiratory infection in a double-blind, randomized, controlled trial finding that a dietary zinc supplement resulted in a significant reduction in

respiratory morbidity in preschool children. Interventions to improve zinc intake may improve the health and survival of children in developing countries. However, these prior art reference do not disclose combining micronutrients with prebiotics and probiotics in a lipid-based excipient, in order to provide a composition which is readily administrable on addition to food.

Another factor critical to the immune system is the prevention of infection of the gastrointestinal (GI) tract. The GI tract is a dynamic and integrated ecosystem composed of an organized matrix of host cells, a fully functional immune system and numerous microbial habitats normally colonized by a diverse array of commensal bacterial species. Indigenous non-pathogenic (non-harmful) gut bacteria occupying intestinal habitats provide the front line of mucosal defense against infection. Normal gut bacteria directly prevent intestinal colonization of pathogenic (potentially harmful) organisms by competing more successfully for essential nutrients or for epithelial attachment sites. Through the production of antimicrobial compounds, volatile fatty acids and chemically modified bile acids, indigenous gut bacteria also create a local gut environment that is unfavorable for the growth of most enteric pathogens. Indeed, all animals have, and seemingly require, long-term cooperative associations with commensal bacteria in the GI tract.

During the birth process and rapidly thereafter, microbes from the mother and surrounding environment colonize the GI tract. Gut bacterial groups then undergo a characteristic succession until a dense, complex, and stable microbiota has developed. Bacterial succession from that time onward, involves microbe-microbe and host-microbe interactions and is dependent on host supplied exogenous and endogenous nutrients. Thus nutritional modulation of the intestinal microbiota critically affects the susceptibility to enteric diseases and likely has long-term effects on immune competence and self-tolerance.

Collins et al.[3] discuss the role played by probiotics, prebiotics, and synbiotics in maintaining the health of the human large intestine, as well as dietary supplementation with probiotics, prebiotics, and synbiotics. However, this prior art reference does not disclose combining micronutrients with prebiotics and probiotics in a lipid-based excipient, in order to provide a composition which is readily administrable on addition to food.

Micronutrient malnutrition can be prevented, or at least controlled, by diet diversification, food fortification and nutrient supplementation. However, these solutions cannot readily be implemented in developing countries. For example, the ability of those in developing countries to diversify their diet is dictated not only by the availability of foods with a high nutrient content, but more importantly by the cost of such foods. Micronutrient-fortified foods are, of course, an appropriate, effective means to prevent malnutrition; however, the cost of these foods is prohibitive to most families living in developing countries, or in developed countries, but who cannot afford these foods.

Although, in an ideal world, the prevention of micronutrient deficiency would be through the ingestion of micronutrient-containing foods, the majority of infants in developing countries live in families where the cost of nutrient dense foods is prohibitive. Thus, alternate strategies must be found, like the use of micronutrient and prebiotic, probiotic and synbiotic supplements. Unlike in older children and adults, infants cannot swallow tablets or pills. Thus, presently, micronutrients such as iron are given in the form of a solution (syrup or drops) supplements for infants and young children. Iron solutions have significant disadvantages compared to tablets or pills, including shorter half life and a higher cost of shipping. more complicated dispensing directions, a higher likelihood of dosage errors, possible staining of teeth (reversible), poor compliance (the strong metallic taste of iron drastically reduces compliance) and

caregiver burnout (infants object to the drops thus caregivers tend to quickly give up.

Vitamin A is often provided via an intramuscular injection or periodic massive doses. Intramuscular injections are painful and must be repeated at regular intervals to be effective over the long-term. Periodic dosing can cause temporary vitamin A toxicity if the dose is very high, but more importantly, periodic dosing is quite expensive since a health care worker administers the dose. There are advantages to provide low dose micronutrients such as vitamin A on a daily basis, such as the lower cost of delivery, potentially better absorption of low doses repeated frequently than large doses provided infrequently, a more efficient delivery system since the sachet is used in homes by parents without the need for health care workers.

The use of excipients such as waxes and lipids is well known in the art, for example U.S. patent 4,882,167 to Jang, which is incorporated by reference, teaches a hydrophobic carbohydrate polymer and a wax, fatty acid material or neutral lipid for use in a controlled release dosage form. U.S. patent 5,162,057 to Akiyama, which is incorporated by reference, discloses coating agents such as fatty acid esters of polyglycerols, which may optionally include waxes.

A composition containing lipid-coated micronutrients and the use of this composition to coat food products during the manufacturing process is taught in U.S. patent 3,992,556 to Kovacs, which discloses mixing a micronutrient with a melted fat carrier and then applying the fat carrier/food supplement mixture to the surface of a pre-made food product and cooling the food product below the melting point of the fat. Kovacs also discloses a toasting or heating process by which the layer of food supplement is attached to food products such as breakfast cereals, crackers, cookies, potato chips, and similar snack foods, flour and pasta. Kovacs teaches the addition of the food supplement/fat carrier mixture during the food manufacturing process.

However, such a composition cannot be readily administered to food by the eventual consumer.

In my Canadian patent application 2,230,801, which is incorporated by reference, a composition containing microencapsulated iron granules in combination with a lipid-based excipient is described. The composition may be added to food by the consumer, and can be used with liquid foods. However, this application does not disclose supplementation with micronutrients other than iron, nor does it disclose the use of prebiotics, probiotics or synbiotics to stimulate the non-pathogenic bacterial populations of the gastrointestinal tract.

Accordingly, there is a need for a composition which combines micronutrient supplementation with supplements for stimulation of the non-pathogenic bacterial populations in the GI tract, in order to improve general immunity. Such a composition is needed in an inexpensive form which can be easily added to food and is suitable for use with infants or young children.

Summary of the Invention

It is an object of the present invention to provide a composition for enhancement of general immunity in a mammal, which combines micronutrient supplements, prebiotics, probiotics, or synbiotics. The present composition advantageously provides the micronutrients in combination with one of a prebiotic, probiotic, and synbiotic in an inexpensive form which is readily administrable on addition to food. The micronutrients may be micro-encapsulated.

It is another object of the present invention to provide a composition containing micronutrients in combination with prebiotics, probiotics or synbiotics, which may be sprinkled directly on to foods, which is inexpensive to manufacture and has a long shelf life.

It is a further object of the present invention to provide a composition with numerous advantages over old methods of micronutrient and prebiotic or probiotic supplementation. When added to food, the composition does not change the colour or texture of the food. The taste of infant cereals is not affected, at least from an adult perspective.

In a preferred embodiment, the composition is provided in single-dose sachets which are simple to use, reduce wastage and reduce the likelihood of an accidental overdose from the ingestion of too much micronutrient.

According to an aspect of the present invention, there is provided a composition useful for enhancing general immunity comprising: at least one micronutrient in a bio-available form; one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and a pharmaceutically acceptable excipient selected from the group of a lipid-based excipient, and a carbohydrate-based excipient.

According to another aspect of the invention, a use of the above composition is provided, wherein a therapeutically effective amount of the composition is added to food to be administered to a mammal.

According to a further aspect of the invention, there is provided a process for producing a composition useful for enhancing the general immunity of a mammal which consists essentially of the step of combining a micronutrient in a bio-available from with one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic and a pharmaceutically acceptable excipient selected from a group of a lipid-based excipient and a carbohydrate-based excipient.

According to yet another aspect of the invention there is provided a method for

enhancing the general immunity of a mammal comprising the steps of removing a composition comprising micro-encapsulated micronutrient granules, a substance selected from the group of a prebiotic, probiotic or synbiotic, and a pharmaceutically acceptable excipient selected from the group of a lipid-based excipient and a carbohydrate-based excipient, from packaging material; adding a therapeutically effective amount of said composition to a food; and administering the food to said mammal.

According to a further aspect of the invention there is provided a method of treating iron deficiency anemia in children comprising administering a composition comprising microencapsulated iron in a bioavailable form; one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and a pharmaceutically acceptable excipient selected from the group of a lipid-based excipient and a carbohydrate-based excipient.

According to a further aspect of the present invention, there is provided an article of manufacture including packaging material and a pharmaceutical composition contained within said packaging material which is effective to enhance general immunity. The composition comprises one or more micronutrients, one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and pharmaceutically acceptable excipient selected from the group of a lipid-based excipient and a carbohydrate-based excipient.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will

become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

FIGURE 1 is a bar graph illustrating the effect of various iron-containing compositions on hemoglobin response in rats.

Detailed Description of the Invention

The term "enhance general immunity" or "enhancing general immunity" as it is used herein refers to the development of an effective immune system through enhancement of the natural immunosurveillance immune effector mechanism. The enhancement could be mediated by either increasing the frequency and absolute number of effector cells that carry out such function or by enhancement of the cellular mechanisms by which such effector cells mediate their function. In addition, the term "enhance general immunity" or "enhancing general immunity" as it is used herein also refers to the growth of non-pathogenic bacteria in the gut in order to provide the front line of mucosal defense against infection, prevent intestinal colonization of pathogenic organisms, and create a local gut environment that is unfavorable for the growth of most enteric pathogens.

The term "micronutrient" as used herein refers to essential dietary nutrients needed by humans in small amounts. Their absence over varying periods of time will result in clinical deficiency syndromes. The preferred micronutrients for the present invention are iron, zinc, iodine, vitamin A, and vitamin C (ascorbic acid).

The term "prebiotic" as used herein refers to a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity or both of one or a limited number of bacterial species already resident in the colon[4]. Preferably,

a prebiotic should also be:

(i) neither hydrolyzed by nor absorbed in the upper part of the intestinal tract;

(ii) a selective substrate for one or a limited number of potentially beneficial commensal bacteria in the colon, thus stimulating the bacteria to grow, become metabolically activated, or both; and

(iii) able, as a consequence, to alter the colon microflora toward a more healthy composition.

Most prebiotics are directed toward the growth of lactic acid-producing organisms because of the positive effect these organisms have on the GI tract. Examples of prebiotics include but are not limited to fructooligosaccharide (FOS) (e. g. oligofructose and neosugar), inulin, glucooligosaccharide (GOS), lactulose, and lactitol.

The preferred prebiotic according to the present invention is FOS. FOS is derived from the chicory plant and is commercially available. Consumption of FOS has been shown to result in numerical predominance of bifidobacteria in feces. The following health advantages have been shown to be associated with bifidobateria in the adult and infant human gut[3]:

(i) inhibition of pathogen growth;

(iv) immunomodulatory activity;

(v) restoration of gut flora after antibiotic therapy;

(vi) production of digestive enzymes;

(vii) positive effects on antibiotic-associated diarrhea; and

(viii) repression of rotaviruses.

The term "probiotic" as used herein refers to a live microbial food supplement that beneficially affects the host animal by improving its intestinal microbial balance. Preferably, the present composition includes a probiotic which:

(i) exerts a beneficial effect on the host;

(ix) is nonpathogenic and non toxic;

(x) contains a large number of viable cells;

(xi) is capable of surviving and functioning in the gut; and

(xii) remains viable during storage and use.

Health advantages associated with probiotic intake include: [6, 7]

(xiii) alleviation of symptoms of lactose malabsorption;

(xiv) increased natural resistance to infectious diseases of the intestinal tract;

(xv) improved digestion; and

(xvi) stimulation of GI immunity.

Examples of probiotics include but are not limited to Lactobacilli (*L. acidophus, L. casei, L. delbrueckii* subsp. *bulgaricus, L. reuteri, L. brevis, L. cellobiosus, L. curvatus, L. fermentum, L. planatarum),* Gram-positive cocci *(Lactococcus lactis subsp. thermophilus, Enterrococcus faecium, S. diaacetylactis, S. intermedius),* Bifidobacteria (*B. bifidium, B. adolescentis, B. animalis, B. infantis, B. longum, B. thermophilum)*

The term "synbiotic" as used herein refers to the combination use of pre-and probiotics[4]. Examples of synbiotics include but are not limited to Bifidobacteria +FOS, Lactobacilli + lactitol, and Bifidobacteria + GOS.

The term "lipid-based", as it is used herein with respect to the excipient, is meant to refer to excipients which are lipids, or which comprise a lipid component. Lipid-based excipients will combine with the microencapsulated micronutrient granules of the present composition in a chemically stable manner in which no adverse interaction occurs such as undesirable aesthetic changes or undesirable changes to the taste of the product. Moreover, lipid-based excipients conveniently allow combination of the composition with foods, the means by which it is administered.

The term "carbohydrate-based" as it is used herein with respect to the excipient, is meant to refer to excipients which are carbohydrates, or which comprise a carbohydrate component. Examples of suitable carbohydrate-based excipients are dextran, corn syrup solids or glucose polymers.

Preferably, the micronutrient is microencapsulated. In a preferred embodiment, one of the micronutrients included in the composition of the present invention, is iron in the form of microencapsulated iron granules. Microencapsulation of iron protects the iron from the food to which it is added. Iron is a potent oxidizing agent. When a soluble form of iron comes in contact with food, it can change the colour, taste and smell of the food. To prevent this from occurring, the iron is encapsulated with a thin soy-lipid coating. The microencapsulated iron granules of the present composition may comprise any bioavailable solid form of iron including iron salts such as ferrous sulphate, ferrous fumarate, ferrous succinate, ferrous gluconate, ferric pyrophosphate, ferric saccharate, ferric orthophosphate or any other compound capable of providing

iron with an appropriate bioavailability. Bioavailability can be determined using the standard "hemoglobin-repletion" method described in detail by Fritz et al.[8] This method generally involves feeding anemic rats with a test iron compound and comparing their iron uptake with the iron uptake of anemic rats fed a reference compound determined to have a relative iron bioavailability of 100%.

In a most preferred embodiment, ferrous fumarate is combined with FeNaEDTA (Iron sodium EDTA). This combination of two iron sources increases the bioavailable iron. Cereals such as maize are high in phytate content, which decreases the absorption of iron. The addition of FeNaEDTA aids in the absorption of the natural iron in the cereal as well as any iron exogenously added to the cereal. The NaEDTA binds the phytate, thus allowing the iron to be absorbed from the proximal small intestine (the duodenum).

The selected iron compound is formed into granules using techniques and machinery well-known to those of skill in the art. For use in the present composition, granules preferably have a diameter of no more than about 850 microns. Granules of this size range can be obtained, for example, using a U. S. No. 20 sieve. The granulated iron compound is provided as a fine free flowing powder.

Once formed into granules of a desired size, the iron compound may be coated or encapsulated with an inert substance that will not interfere with the uptake of the iron compound. The coating functions to sustain the release of the iron, effectively masking the characteristic unpleasant taste of the iron compound, preventing discoloration of the foods to which it is added, thereby providing a form of iron that can readily be added to foods. The coating also prevents the undesirable interaction between nutrients in the foods to which it is added as well as additional nutrients that may be added to the composition itself. The inert coating may be selected from

a number of suitable substances including, but not limited to, mono-or diglycerides, ethyl cellulose, hydrogenated soybean oil, acacia gum and mixtures thereof. Alternatively, iron which is supplied in a microencapsulated form may be used, for example Descote™ ferrous fumarate.

The encapsulated granulated iron compound is admixed with a pharmaceutically acceptable lipid-based or carbohydrate-based excipient. The term "pharmaceutically acceptable" refers to an excipient acceptable for use in the pharmaceutical and veterinary arts, which is not toxic or otherwise unacceptable. Examples of suitable lipid-based excipients include mono-, di- and tri-glycerides, especially naturally extracted unsaturated edible oils in hydrogenated form (such as vegetable oil, castor oil, cottonseed oil, corn oil, canola oil, rapeseed oil, peanut oil, sesame seed oil, coconut oil and mixtures thereof). Examples of suitable carbohydrate-based excipients include dextran. A most preferred excipient contains corn syrup solids, hydrogenated vegetable oil and/or hydrogenated coconut oil, sodium caseinate, potassium phosphate di-basic, sodium phosphate di-basic, mono and diglycerides, acetylated tartaric acid esters of monoglycerides, artificial colour, and natural and artificial flavour.

Further, the absorption of iron is known to be enhanced in the presence of reducing compounds. Examples of reducing compounds are compounds containing sulfhydryl groups such as the amino acids, lysine and histidine. The absorption of iron is also enhanced in the presence of meat. Accordingly, the present composition can advantageously be consumed with meat. Alternatively, the present composition may additionally contain desiccated meat particles to provide enhanced iron absorption and to provide protein content that would be particularly desirable for administration to populations in which protein consumption is low, such as populations in developing countries.

Preferably, the present composition is supplemented with additional micronutrients. Such additional micronutrients may function to enhance the immune system, as well as to enhance the absorption of iron on administration. In a preferred embodiment of the present invention, the composition additionally comprises ascorbic acid (vitamin C), preferably in an amount ranging from about 40-50 mg per 15 mg of elemental iron. The ascorbic acid enhances the absorption of the iron into the bloodstream, providing a more effective composition. Alternatively, or additionally, the present composition may be supplemented with other micronutrients, particularly those micronutrients which are typically absent from the diet or present in insufficient quantities. Examples of micronutrients that may be added to the composition include vitamin A, zinc and iodine, provided in appropriate bioavailable form. In this regard, vitamin A may be added to the present composition in the form of retinyl palmitate or retinol acetate, zinc may be added in the form of zinc sulfate or zinc gluconate, while iodine may be added in the form of potassium iodide. The iodine is preferably coated with dextran, which helps to prevent oxidation.

It will be appreciated that suitable amounts of additional micronutrients will vary with the micronutrient in question. For example, amounts of about 0.35-0.45 mg of retinyl palmitate per 15 mg of elemental iron, about 5-10 mg of elemental zinc per 15 mg of elemental iron and about 0.25-0.5 mg of iodine per 15 mg of elemental iron may appropriately be added to the present composition.

Davidsson[9] recently demonstrated geometric mean [^{57}Fe] ferrous fumarate bioavailability from cereal of 4.1% (range 1.7-14.7%) and 1.3% (range 0.7-2.7%) from [^{57}Fe] ferric pyrophosphate in non-anemic infants. In vitro bioavailability data from our laboratory suggests a range of bioavailability of encapsulated ferrous fumarate of 1.5-3%. If the iron requirement (the amount of 'absorbed' iron) for the non-anemic infant is around 1 mg/day

and 1.5 mg/day for the anemic infant, then the range of doses of oral iron needed to achieve the iron requirement would be as indicated in the table below:

Table 1: Dosage range of oral iron for infants

	dose of oral iron to achieve estimated iron requirement at ..		
Iron Requirement	1.5% absorption	3.0% absorption	4.5% absorption
1.0 mg/day	66.7 mg*	33.3 mg	22.2 mg
1.5 mg/day	100 mg	50 mg	33.3 mg

* calculated as 1.5% of an unknown dose = 1 mg; thus 1.5% x 66.7 = 1 mg

To the micronutrient composition is added a prebiotic, probiotic or synbiotic. Although traditional freeze-dried probiotics can be used, live probiotics are preferred. To protect the probiotic from degrading, the probiotic is preferably encapsulated with wax such as beeswax, carnauba wax, spermaceti, lecithin, paraffin and microcrystalline wax, a carbohydrate such as dextran or most preferably a thin vegetable oil coating. Preferably, a microencapsulated form of probiotic that is heat stable even at high environmental temperatures, such as Probiocap™ is used. Probiocap™ is encapsulated by coating in a matrix of food-grade vegetable fatty acids. In the past, probiotics had to be kept refrigerated in order to maintain the viability of the bacteria culture. Traditional freeze-dried probiotic bacteria are sensitive to high moisture, extreme temperatures and other physical and chemical stresses. This sensitivity limits their use in many applications. Their viability also decreases during digestion due to the extreme gastric acidity which has long been a concern. Probiotic applications, including the provision of probiotics in non-refrigerated sachets, was limited due to elevated temperatures and the presence of oxygen and moisture that would adversely affect survival rates.

It will be appreciated that there is no restriction on the foods or beverages to which the present composition can be added. Since the present composition is particularly beneficial for use in the prevention of anemia in infants and young children, the composition will typically be added to foods and beverages generally consumed by infants and young children. Examples of such foods include pureed or semi-solid foods, for example cereals, gruels, porridges, purees of fruit, vegetables, meat or mixtures thereof, as well as milk-based products including, but not strictly limited to, milk, powdered milk, infant formula, puddings, yogurt, creamed cheese, cottage cheese, and other dairy products which form a part of the diet of infants and young children. The term milk-based products is also meant to include milk substitutes including lactose-free milk and associated products, soy milk and the like.

In a preferred embodiment, a single daily dosage of the composition is packaged, for example in a sachet-type package, comprising about 60 mg of elemental iron in the form of micro-encapsulated granules, prebiotics and probiotics in therapeutically effective amounts, which are known to those skilled in the art , for example, 1 to 2 x 10^6 colony forming units (CFUs) of probiotics and about 400-450 mg of excipient. In a particularly preferred embodiment, the package will additionally include ascorbic acid in an amount of about 20-100 mg, iodine in an amount of 20-100 µg, and vitamin A in an amount of 50-2500 IU.

A method for enhancement of general immunity in a mammal is also provided. The method involves the steps of adding a therapeutically effective amount of the present composition to a food, and then administering the food to the mammal requiring treatment. The term "therapeutically effective" as it is used with respect to the present composition refers to an amount which is effective to prevent iron deficiency anemia, or at least minimize the occurrence of adverse effects related thereto, while not exceeding an amount which would be toxic or otherwise harmful. In this regard, precise dosage sizes appropriate to prevent anemia can readily be established in

appropriately controlled trials.

The present invention is described in more detail by reference to the following specific examples which are not to be construed as limiting.

Example 1 - Preparation of an Iron-containing Composition

Encapsulated ferrous fumarate 60% (1 gram delivers 600 mg ferrous fumarate) (Descote® Ferrous Fumarate 60), having a particle size of no more than about 850 microns in which about 99% of the particles pass through a U. S. No. 20 sieve, was obtained from Particle Dynamic Inc, St. Louis, MO.

Ascorbic acid (3.5 kg; obtained from Basf) was thoroughly mixed in a large aluminum bowl with an excipient (25 kg; obtained from New Dundee Creamery, Division of Ault Foods Limited) containing corn syrup solids, hydrogenated vegetable oil and/or hydrogenated coconut oil, sodium caseinate, potassium phosphate di-basic, sodium phosphate di-basic, mono and diglycerides, acetylated tartaric acid esters of monoglycerides, artificial colour, and natural and artificial flavour.

In a 2-stage fill, 200 mg aliquots of encapsulated ferrous fumarate were added to foil-lined sachet packets followed by the addition of 300 mg of ascorbic acid/excipient mixture. The sachets were appropriately sealed along their open edge.

Optionally, 2.1 kg zinc gluconate is admixed with the ascorbic acid and excipient. This mixture is then added to ferrous fumarate-containing sachets as set out above.

Example 2 -Relative Bioavailability of Micro-encapsulated Iron

The bioavailability of iron in the composition set out in Example 1 has been determined using the hemoglobin-repletion test in rats as follows.

Male weanling Sprague-Dawley rats housed individually in stainless steel cages were fed a low iron diet and de-ionized distilled water ad lib for 24 days. The low-iron diet contained no more than about 3 mg of iron per kg of diet. Following the 24 day depletion period, approximately 200 µl of blood was drawn from the tail vein of each rat for hemoglobin analysis. Anemic rats having hemoglobin values between 30 and 60 g/L were used in the study. The rats were housed individually in cages in a randomized block design. The rats were divided into groups, each group being fed ad libitum a test diet selected from 0, 10 or 20 mg of one of microencapsulated or coated ferrous fumarate (prepared as described in Example 1), microencapsulated or coated ferrous fumarate with zinc, uncoated ferrous fumarate particles or uncoated ferrous sulphate (a reference compound determined to have a relative bioavailability of 100) per kilogram of diet. The test groups are more specifically set out in the following Table 2:

Table 2: Bioavailability Test Groups

# of Animals	Ferrous Sulfate (Fe SO47H20)	Coated Ferrous Fumarate	Coated Ferrous Fumarate+Zinc	Ferrous Fumarate
10	0	0	0	0
10	10 mg Fe/kg diet	0	0	0
10	20 mg Fe/kg	0	0	0

	diet			
10	0	10 mg Fe/kg diet	0	0
10	0	20 mg Fe/kg diet	0	0
10	0	0	0 Fe; 10 mg/kg Zn	0
10	0	0	10 Fe; 10 mg/kg Zn	0
10	0	0	20 Fe; 10 mg/kg Zn	0
10	0	0	0	10 mg Fe/kg diet
10	0	0	0	20 mg Fe/kg diet
Total 100				

The results, as shown in Figure 1, indicate that hemoglobin response is dependent on the amount of iron in the rat's diet. Moreover, there was no significant difference in the hemoglobin response between rats fed similar amounts of iron as the reference compound (ferrous sulfate) versus rats fed micro-encapsulated ferrous fumarate.

Referring to Fig. 1, the control group represents rats fed a diet containing no iron, the "low iron" diet represents a diet containing 10 mg micro-encapsulated ferrous fumarate/kg of diet, the "high iron control" diet represents a diet containing 20 mg ferrous sulfate/kg of diet and the "high iron" diet represents a diet containing 20 mg

micro-encapsulated ferrous fumarate/kg of diet. There was no change in the hemoglobin of the control after 14 days of feeding, while mean hemoglobin response of the low iron diet group was 18 g/L and the mean hemoglobin response of the high iron control and high iron diet groups was 31 g/L and 33 g/L, respectively.

Example 3 – Preparation of sachets containing 80mg iron and 50 mg ascorbic acid

For an 80 mg dose of iron, and a 50mg dose of ascorbic acid, the following ingredients were supplied as described in Example 1 and put together and mixed thoroughly. Sachets were filled as a single fill, with 1000 1 gram sachets obtained per kg of raw materials.

Dose	Ingredient	g/kg	g/sachet
80 mg	Descote Ferrous Fumarate	405.60	0.4056
40 mg	Ascorbic acid (10% overage)	50.00	0.05
	Excipient	544.40	0.5444
	TOTAL	1000.00	1.00

Example 4 - Randomised controlled trial of microencapsulated ferrous fumarate sprinkles and ferrous sulphate drops for treatment of anaemia in Ghanaian infants and young children

The effectiveness of the composition of example 3 (referred to as "sprinkles") in treating anaemia in infants and young children has been determined as follows:

In a randomised controlled trial, 837 children (age range 6–24 months; haemoglobin values 70–99 g/L) were studied. The children were selected from rural villages of

Kintampo, Ghana, a malaria-endemic area. One treatment group (n=280) received a daily sachet as described in example 3, containing microencapsulated ferrous fumarate (80 mg elemental iron) plus ascorbic acid; another (n=280) ferrous sulphate drops once daily (40 mg elemental iron); and the control group (n=277) ferrous sulphate drops three times per day (total dose 40 mg elemental iron). Treatment lasted for 2 months. Haemoglobin and serum ferritin values were measured at baseline and 2 months later.

Field workers visited infants at 2-week intervals after the baseline visit, for a total of 5 visits. During the baseline assessment, a written questionnaire was administered to collect demographic, nutritional, and health data for each infant. During the final visit and each of the 2-week visits, a questionnaire about the side effects and compliance over the preceding 7 days (eg, a question about how often the child received drops in the last 7 days) was completed. Data collected about side effects included the incidence of diarrhea, constipation, and general discomfort after ingestion of the coated iron or iron drops. Questions about adherence to treatment included whether the children objected to taking the iron and whether microencapsulated ferrous fumarate changed the colour or consistency of the infants' food. Fieldworkers provided parents with oral educational reinforcement to maximize adherence to the treatment.

Anthropometric measurements, including weight for age, height for age, and weight for height for age, were completed during baseline and final visits. An infant-length board with a sliding foot-board was used for measurement of the child's body length, and a hanging scale graduated in 100-g divisions, for weight measurements. Two fieldworkers completed the measurements in duplicate using standardized techniques.

Capillary blood samples at baseline and final visits were obtained from a finger prick using aseptic techniques, and haemoglobin was measured on the spot with portable

Hemocue photometers (Hemocue Inc, Angelholm, Sweden). Malaria parasite smears were taken (at baseline only), and 500 µL blood samples were collected and preserved in ice-lined cold boxes. Blood samples were returned to the base station within 6 hours of collection, where the serum was separated by centrifugation (10 minutes at 1300 RPM) before storage at -40^0C. Serum ferritin was assayed in duplicate by a commercial enzyme-linked immunosorbent assay (ELISA), using a Spectro Ferritin Kit (Ramco Laboratories, Houston, TX). [14] Baseline and final ferritin samples from an individual subject were assayed on the same day (in a single batch) on one 96-well microtitre plate to minimise inter-assay variation. An external reference standard (Lyphochek Anaemia Control, Bio-Rad, Anaheim, CA) was assayed in duplicate on each microtitre plate for the ferritin assay.

The blood films were stained and examined for malaria parasites at the end of the study. Children whose blood films indicated a possible malaria infection were treated at home for malaria. Children who were severely anaemic (haemoglobin <70 g/L) were excluded from the trial and treated.

Those children in the positive control group were given ferrous sulphate drops (5 mg/kg/day of elemental iron, rounded to a total of 40 mg of elemental iron) provided in three equal doses per day. The other two groups received either the ferrous sulphate drops (40 mg) provided daily in a single bolus or microencapsulated ferrous fumarate (80 mg of elemental iron) in the composition of Example 3 packaged in a sachet with ascorbic acid (50 mg), called sprinkles sachets, and added to the child's meal serving (after it was cooked) once daily.

Children were individually randomised to one of the three treatment groups. Randomisation was done with sealed opaque envelopes containing group designations, which were generated randomly by computer with Microsoft Access 97 (Microsoft Corporation, Seattle, WA). It was not feasible to blind the field staff or the

mothers to the group to which the children were assigned. However, the persons responsible for the laboratory and data analyses were blinded to the group designations.

Haemoglobin

In all groups, there was a significant increase in haemoglobin concentrations from baseline to the end of the study ($p<0.001$; table 1). The change in haemoglobin concentrations (from baseline to final) was similar among treatment groups. Successful treatment of anaemia (hemoglobin > 100 g/L) occurred in 58% in the sprinkles group, in 61% of the once-a-day drops group, and in 56% of the control group. There was no significant difference between these ($p=0.51$). Ferritin levels increased significantly in each group ($p<0.001$) although less so in the sprinkles group. The relative risk of remaining anaemic after 2 months of treatment was 1.03 times greater for the sprinkles group (95% CI 0.88–1.20, $p=0.75$) and 0.92 times lower for once-daily ferrous sulphate group (95% CI 0.79–1.06, $p=0.26$) than that for the three-times-daily ferrous sulphate group, but the differences were not significant. Infants who were positive for malaria were more likely to be anaemic at the end of two months in all groups. The relative risk of remaining anaemic after 2 months of treatment was 1.23 times greater for those with malaria (95% CI 1.10–1.37, $p=0.0006$) than those who were malaria-free. The mean haemoglobin values are set out in the following table.

Table 3: Mean haemoglobin (g/L) by treatment group at baseline and after 2 months

	Treatment group		
Haemoglobin values (g/L)	Sprinkles	Drops once daily	Drops 3x/day
Mean (SD) at Baseline	87 (8)	88 (8)	87 (8)

Final	102 (16)	102 (18)	100 (17)

Ferritin

The geometric mean ferritin values at baseline were similar in all groups (table 2). There was a significant increase (around two-fold or more) after 2 months of treatment ($p<0.0001$); however, the values in both drops groups were significantly higher than those in the sprinkles group. The variance for ferritin values was quite wide at both baseline and the end of the study as is usual with the wide inter-individual and analytic variance associated with this measure. The ferritin values are set out in the following table.

Table 4: Geometric mean ferritin values (µg/L) by treatment group at baseline and after 2 months of intervention

	Treatment group		
Ferritin values (µg/L)	Sprinkles	Drops once daily	Drops 3x/day
Baseline	42.9 (99.4)	34.8 (74.8)	45.5 (83.5)
Final	81.1 (140.9)*	102.1 (113.2)	111.0 (132.1)

Data are geometric means (SD); analysis was done with log-transformed values since ferritin values are not normally distributed. Mean ferritin increased significantly from baseline to the final visit in all treatment groups ($p<0.001$). Mean ferritin value at final sampling was significantly lower ($p<0.05$) from both drops groups. Normal range for ferritin is 12 – 80µg/L for infants 6 to 24 months of age (23)

Anthropometric measurements

At baseline all infants had similar weight for age z-scores (mean [SD] –1.48 [1.10]), height for age (-1.36 [1.12]) and weight for height (-0.74 [0.94]). No effect of

treatment was found for weight for age and weight for height for age. There was a significant decrease in height-for-age z-scores in all three groups from baseline to the final measurements (mean [SD] change –0.16 [0.79], p<0.001). The anthropometric measurements are set out in the following table.

Table 5: Anthropometric measurements for each treatment group at baseline and after 2 months of intervention

Anthropometric measure	Treatment groups			
	Sprinkles	Drops once daily	Drops 3x/day	Combined*
Weight-for-age z-score				
Baseline	–1.49 (1.05)	–1.54 (1.15)	–1.38 (1.09)	–1.48 (1.10)
Final	–1.53 (0.97)	–1.53 (1.13)	–1.42 (1.06)	–1.49 (1.06)
P	0.26	0.78	0.26	0.14
Height-for-age z-score				
Baseline	–1.32 (1.07)	–1.44 (1.14)	–1.38 (1.09)	–1.36 (1.12)
Final	–1.47 (1.04)	–1.60 (1.28)	–1.53 (1.14)	–1.53 (1.16)
P	0.0001	0.025	0.0003	<0.0001
Weight-for-height-for-age z-score				
Baseline	–0.80 (0.90)	–0.74 (0.97)	–0.66 (0.93)	–0.74 (0.94)
Final	–0.81(0.93)	–0.66 (0.88)	–0.58 (0.95)	–0.68 (0.92)
P	0.97	0.11	0.16	0.095

Data are mean (SD). *Defined as the mean for all treatment groups.

Example 5 – Composition containing Microencapsulated Iron and Vitamin A

500 mg sachets each containing a 40 mg dose of iron and a 2000 IU dose of vitamin A as retinol acetate (supplied by Hoffmann-La Roche) were prepared by mixing the

following ingredients. The descote ferrous fumarate, ascorbic acid and excipient were supplied as described in Example 1.

Dose	Ingredient	g/kg	g/sachet
40 mg Fe	Descote ferrous fumarate	405.60	0.2028
50 mg	Ascorbic acid	100.00	0.05
2000 IU	Retinol Acetate	6.72	0.00336
	Excipient	487.68	0.24384
	TOTAL	1000.00	0.5

2000 sachets per kg of composition each containing 0.5g were filled as a single fill.

Example 6: Composition containing microencapsulated iron

500 mg sachets each containing a 40 mg dose of iron were prepared by mixing the following ingredients. The descote ferrous fumarate, ascorbic acid and excipient were supplied as described in Example 1.

Dose	Ingredient	g/kg	g/sachet
40 mg	Descote ferrous fumarate	405.60	0.2028
50 mg	Ascorbic acid	100.00	0.05
	Excipient	494.40	0.2472
	TOTAL	1000.00	0.5

2000 sachets per kg of composition each containing 0.5g were filled as a single fill.

Example 7: -Randomized controlled trial to determine the effect of frequency of dosing and form of iron on the treatment of iron deficiency anemia.

437 infants (8 to 18 months ± 2 weeks) who 'graduated' from the study as described in Example 4 without iron deficiency anemia (hgb > 99 g/L) were recruited into this

randomized double-blind controlled trial to determine the efficacy of micro-encapsulated ferrous fumarate retinyl palmitate sprinkles in preventing iron deficiency anemia and vitamin A deficiency when used daily in weaning foods. Parents were approached to enroll their infants in the study at the end of the study described in Example 4. After informed consent from the parent(s) was received, infants were randomized to one of the 4 groups (if the hemoglobin is < 100 g/l). The positive control group was given ferrous sulfate drops. The negative control group received the a placebo sachet containing 0.5 grams of excipient (described in Example 1) with a small amount of brown rice.with periodic vitamin A supplements, while the third group received the iron and vitamin A sachets described in Example 5 and the fourth group received iron alone sachets as described in Example 6.

All parents were given the same simple instructions on daily use of sachets (ie "EMPTY THE CONTENTS OF ONE PACKET (SACHET) ON TO THE BABY'S FOOD (cereal, etc). ONLY USE ONE PACKET EACH DAY"). Each family met with a research assistant at least monthly for 6 months. At each visit, a questionnaire was administered with questions concerning general health, food intake, compliance and ease of use of the sachets (see data management manual for the questionnaires that will be used in the study). Infants also had their length, weight and head circumference measured at each visit. At each visit enough sachets were distributed to last until the next visit. A second blood sample (200 -300 µl) was drawn at the end of the study to assess haematologic and vitamin A status (as previously described).

Vitamin A End-Point

If a serum retinol concentration less than 0.7µmol/l is detected, no specific individual therapy is recommended unless clinical signs of deficiency are concurrently detected. The WHO/UNICEF/IVACG Task Force on vitamin A supplements recommends this policy.

Statistical Analysis

The principal outcomes were the efficacy of iron-vitamin A sprinkles in the prevention of anemia (Groups A, B and C versus D; B vs C); and prevention of vitamin A deficiency (Groups B versus A and C). To determine if there is a difference in the proportion of infants with anemia treated with iron from drops or sachets, groups B and C were compared to group A. Analysis was completed by comparing the proportion of infants from each group reaching end points using Chi-square analysis. Secondary outcomes included: (i) the effect of age on prevalence of anemia by group; and (ii) the effect of group on hemoglobin response. Logistic regression and survival analysis was also completed on outcome data. The table below shows the comparisons.

Table 6: Study Groups

Efficacy Study	Group	Statistical Comparisons
Iron drops - +ve control	A	A, B, & C vs D
Iron + Vitamin A (sachet)	B	B vs A &C
Iron Alone (sachet)	C	B& C vs A
Excipient only (sachet) –ve control	D	A, B, & C vs D

Example 8: Composition containing microencapsulated iron and zinc

1 g sachets each containing a 40 mg iron and 10 mg zinc dose were prepared as described in the preceding examples, containing the following ingredients, supplied as described in the preceding examples.

Dose	Ingredient	g/kg	g/sachet
40 mg Fe	Descote ferrous fumarate	405.60	0.2028
10 mg Zn	Zinc gluconate dihydrate	76.10	0.0761
50 mg	Ascorbic acid	50.00	0.05
	Excipient	468.30	0.4683
	TOTAL	1000.00	1.0

1000 sachets per kg of composition each containing 1g were filled as a single fill.

Example 9 – Composition Containing Micronutrients and Probiotics

0.5 g sachets were prepared with the following ingredients

L. rhamnosus + L. acidophilus (encapsulated, Supplied by Institut Rosell-Lallemand Inc., Montreal)	cfu 2×10^6
FOS(supplied by Institut Rosell-Lallemand Inc., Montreal)	40 mg/sachet
Vitamin C	50 mg
Vitamin A (as acetate)	3000 IU
Descote ferrous fumarate)	60 mg
Iron (as ferric sodium EDTA) (Lohmann Inc., Emmerthal, Germany)	2.4 mg
zinc gluconate	10 mg

Example 10 – Pilot Study to Determine the Efficiency of the Composition Containing Micronutrients and Probiotics in Reducing the Incidence and Prevalence of Diarrhea

The composition of example 9 is tested in a double-blind, randomized control trial to investigate the efficiency of the composition of zinc, iron, ascorbic acid and vitamin A and probiotics in reducing the incidence and prevalence of diarrhea. Infants receive one of 3 interventions, described below. The compositions are sprinkled over or

added to infant weaning foods after the foods have been cooked. It is not a component of the food before the food is cooked. It is not added to the food during the cooking process.

Table 7. Intervention Groups

'Sprinkles' Ingredient	Intervention Group		
	Fe Sprinkles (control) n=314	Fe + Zn Sprinkles n=314	Fe + Zn + Pro-b Sprinkles as in example 8 n=314
Iron (as ferrous fumarate and FeNa EDTA) mg	60	60	60
Ascorbic Acid (mg)	40	40	40
Retinol Acetate (IU)	2000	2000	2000
Zinc	-	10	10
Probiotic (CFU)	-	-	$1\text{-}2 \times 10^6$

Therefore all three treatment groups contain the standard sprinkles ingredients; iron, vitamin C and A. To one group zinc is added to the sachets and to another group, zinc and probiotics. This allows us to compare the rates of diarrhea among the three groups.

There is no true placebo group. In order to recruit a representative sample population of infants into the study, anemic as well as non-anemic infants enter the study. A secondary outcome measure in this study is hemoglobin values. Hemoglobin can be directly determined in the field using Hemocues™. Therefore, we identify those infants that are anemic (hemoglobin < 10 g/dL). It would be unethical to randomize infants with anemia into a placebo group where they would not be provided with iron supplementation. Therefore all infants in the study will receive iron.

Infants who are 6 to 18 months of age at time of recruitment, ingesting a weaning food in addition to breast milk, hemoglobin >6.9 g/dL, whose family expects to remain in the vicinity (town, hospital clinic area, etc for 8 months), and who have parental consent are included in the study. Infants <5.5 months of age, sickle cell disease, or with hemoglobin < 7.0 g/dL (infants with severe anemia are referred for evaluation and treatment) are excluded.

The primary outcome measures are incidence and prevalence of diarrhea (the number of new episodes of the illness and the days with the illness, respectively, per total days of observation). A day of diarrhea was defined as a 24 hour period with 3 or 4 unformed stools. An episode of diarrhea was defined as at least 1 day of diarrhea, with the final day of the episode being the last day meeting the diarrhea definition followed by at least 48 hours without diarrhea. An episode of dysentery was defined as an illness meeting the definition of diarrhea in which blood was observed in the stools. An episode of persistent diarrhea was defined as diarrheal illness that lasted >= 14 days.

The secondary outcomes measures include hemoglobin concentration, plasma zinc concentration, anthropometry (weight for height, age for height Z scores), and general health and morbidity data.

Infants are randomly assigned to one of the 3 intervention groups. The infants receive their assigned sprinkles sachet daily for a period of 12 months. Morbidity surveillance field workers visit the child at home weekly to deliver sachet supplies and record information on the number of diarrheal stools, consistency of stools, fever, vomiting, feeding history and compliance data. At baseline and final visit at 12 months, anthropometry measurements and hemoglobin values are assessed. A small capillary blood sample (500 µL) is collected at baseline and final visits to determine plasma zinc concentration.

Analysis of variance is used to look at differences in the incidence and prevalence of diarrhea between groups. Paired t-tests are used to assess change in anthropometry, plasma zinc and hemoglobin over time. Anthropometric measures are converted to z-scores using the NCHS reference standards. Analysis is conducted using SAS 6.12 (SAS Institute, Inc., Carey, NC). The acceptable level of statistical significance for all tests is $p<0.05$.

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I CLAIM:

1. A composition useful for enhancing general immunity comprising: at least one micronutrient in a bioavailable form; one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and a pharmaceutically acceptable excipient selected from the group of a lipid-based excipient and a carbohydrate-based excipient.

2. The composition of claim 1, wherein the at least one micronutrient is selected from the group of iron, iodine, vitamin A, and zinc.

3. The composition of claim 2, wherein the composition comprises iron, vitamin A, and zinc.

4. The composition of claim 2, wherein the composition comprises iron, iodine, vitamin A, and zinc.

5. The composition as in any of claims 3 and 4 wherein the composition additionally comprises FeNaEDTA.

6. The composition of claim 1 wherein the pharmaceutically acceptable excipient is a lipid-based excipient.

7. The composition of claim 1 wherein the pharmaceutically acceptable excipient is a carbohydrate-based excipient.

8. The composition as in any of claims 1-7, wherein the prebiotic is selected from at least one member of the group consisting of FOS, inulin, GOS, lactulose, and lactitol.

9. The composition of claim 8, wherein the FOS is selected from the group of oligofructose and neosugar.

10. The composition as in any of claims 1-7, wherein the probiotic is selected from at least one member of the group consisting of Lactobacilli, Gram-positive cocci, and Bifidobacteria.

11. The composition of claim 10, wherein the Lactobacilli is selected from at least one member of the group consisting of *L. acidophus, L. casei, L. delbrueckii subsp. bulgancus, L. reuteri, L. brevis, L. cellobiosus, L. curvatus, L. fermentum, L. planatanrm.*

12. The composition of claim 10, wherein the Gram-positive cocci is selected from at least one member of the group consisting of *Lactococcus lactis subsp. thermophilus, Enterrococcus faecium, S. diaacetylactis, S. intermedius.*

13. The composition of claim 10, wherein the Bifidobacteria is selected from at least one member of the group consisting of *B. bifidium, B. adolescentis, B. animalis, B. infantis, B. longum, B. thermophilum.*

14. The composition as in any of claims 1-13, wherein the synbiotic is selected from at least one member of the group consisting of Bifidobacteria + FOS, Lactobacilli + lactitol, and Bifidobacteria + GOS.

15. The composition as in any of claims 1-14, wherein at least one micronutrient is microencapsulated with a compound selected from the group consisting of monoglycerides, diglycerides, ethyl cellulose, hydrogenated soybean oil and mixtures thereof.

16. The composition of claim 15 where the probiotic is encapsulated with one or more compounds selected from the group of waxes, carbohydrates, and lipids.

17. The composition of claim 16 where the probiotic is encapsulated with a lipid.

18. A process for producing a composition useful for enhancing the general immunity of a mammal which consists essentially of the step of combining a micronutrient in a bio-available form with one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic and a pharmaceutically acceptable excipient selected from a group of a lipid-based excipient and a carbohydrate-based excipient.

19. A method for enhancing the general immunity of a mammal comprising the steps of:

a) removing a composition comprising microencapsulated micronutrient granules, a substance selected from the group of a prebiotic, probiotic or synbiotic, and a pharmaceutically acceptable excipient selected from the group of a lipid-based excipient and a carbohydrate-based excipient from packaging material;

b) adding a therapeutically effective amount of said composition to a food; and

c) administering the food to said mammal.

20. Use of the composition of any of claims 1-17 for enhancing the general immunity of a mammal.

21. Use of the composition of claim 20, wherein a therapeutically effective amount of the composition is added to food to be administered to the mammal.

22. An article of manufacture including packaging material and a pharmaceutical composition contained within said packaging material which is effective to

enhance general immunity, wherein the pharmaceutical composition comprises one or more micronutrients, one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and pharmaceutically acceptable excipient selected from the group of a lipid-based excipient and a carbohydrate-based excipient.

23. A method of treating iron deficiency anemia in children comprising:

a) removing a composition comprising iron in a bioavailable form; a substance selected from the group of a prebiotic, probiotic or synbiotic, and a pharmaceutically acceptable excipient selected from the group of a lipid-based excipient and a carbohydrate-based excipient from packaging material;

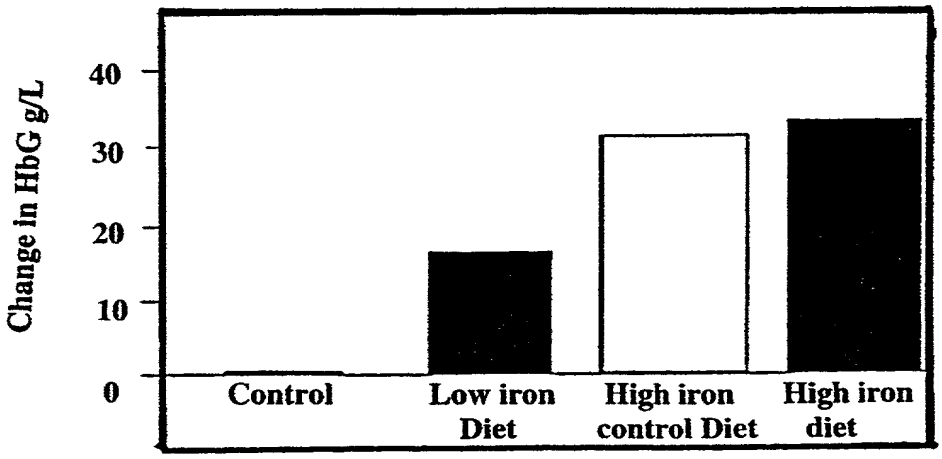
b) adding a therapeutically effective amount of said composition to a food; and

c) administering the food to said mammal.

24. The method of claim 23 where the iron is microencapsulated with a compound selected from the group consisting of monoglycerides, diglycerides, ethyl cellulose, hydrogenated soybean oil and mixtures thereof.

25. The method of claim 24 wherein the composition additionally comprises a micronutrient selected from the group of iodine, vitamin A, and zinc.

Figure 1


Change in HbG g/L
40
30
20
10
0
Control
Low iron Diet
High iron control Diet
High iron diet

COMBINED DECLARATION AND POWER OF ATTORNEY FOR UTILITY PATENT APPLICATION	Attorney's Docket No. 033312-001

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (if only one name is listed below) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (if more than one name is listed below) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

COMPOSITION COMPRISING MICRONUTRIENTS IN COMBINATION

WITH PREBIOTICS, PROBIOTICS, AND/OR SYNBIOTICS

the specification of which

(check one) ☐ is attached hereto;

☒ was filed on August 28, 2000 as

International Application No. PCT/CA00/00990

and was amended on _____; (if applicable)

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE;

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than twelve months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code Sec. 119 and/or Sec. 365 of any foreign application(s) for patent or inventor's certificate as indicated below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application(s) on which priority is claimed:

COMBINED DECLARATION AND POWER OF ATTORNEY	Attorney's Docket No. 033312-001

COUNTRY/INTERNATIONAL	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
Canada	2,281,463	26 August 1999	YES X NO__
			YES__ NO__

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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